Immunohistochemistry in Neurosurgery for Pathological Diagnosis: A Case Illustration of a Sellar Tumor

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Immunohistochemistry is an essential clinical and research tool in medical science. It helps in making specific diagnosis by detecting the lesion specific markers. It also helps in predicting the final outcome of a neoplastic lesion by detecting various prognostic markers.

Thirty four years old male with a huge sellar and suprasellar lesion with cystic and solid component presented with bitemporal hemianopia and 3rd ventricle compression and hydrocephalus. Provisional diagnosis of non-functioning pituitary adenoma with a differential diagnosis of craniopharyngioma was made. Tumor was excised by craniotomy and the specimen sent for histopathological diagnosis. The report showed confusing result mentioning the presence of signs of pituitary adenoma and low grade astrocytoma.

Glial fibrillary acidic protein (GFAP), a marker of astrocytic tumor and Ki-67 (MIB-I), a marker of cellular proliferation in neoplastic lesion were investigated by immunohistochemistry to confirm the diagnosis. The indirect method of immunostaining, LSAB (Labeled Strept Avidin Biotin method), was employed using the histofine SAB kit. Immunostaining showed absence of GFAP positive cells and about 1% of MIB-I labeling index suggesting the tumor was not of astrocytic origin and thus was pituitary adenoma. Lower expression of MIB-I was also in the favor of pituitary adenoma.

The final diagnosis of pituitary adenoma was made by excluding the possibility of astrocytoma. Thus immunohistochemistry is helpful in such situation. The histopathological evaluation of any neoplastic lesion is not complete without immunohistochemistry.

Key words: astrocytoma, GFAP, immunohistochemistry, MIB-I, pituitary adenoma

Immunohistochemistry, as is well known, is an essential tool in the field of medical pathology in this era. It not only helps in the pathological diagnosis of a lesion but also helps in detection of different tumor markers and predicts the prognosis on that basis. The staining technique of immunohistochemistry is called immunostaining. Thus, immunostaining has a great role in clinical field as well as in the experimental and research field of medicine.

Having said that, immunostaining is regarded as a high technology, despite its simplicity, and is not yet a routine

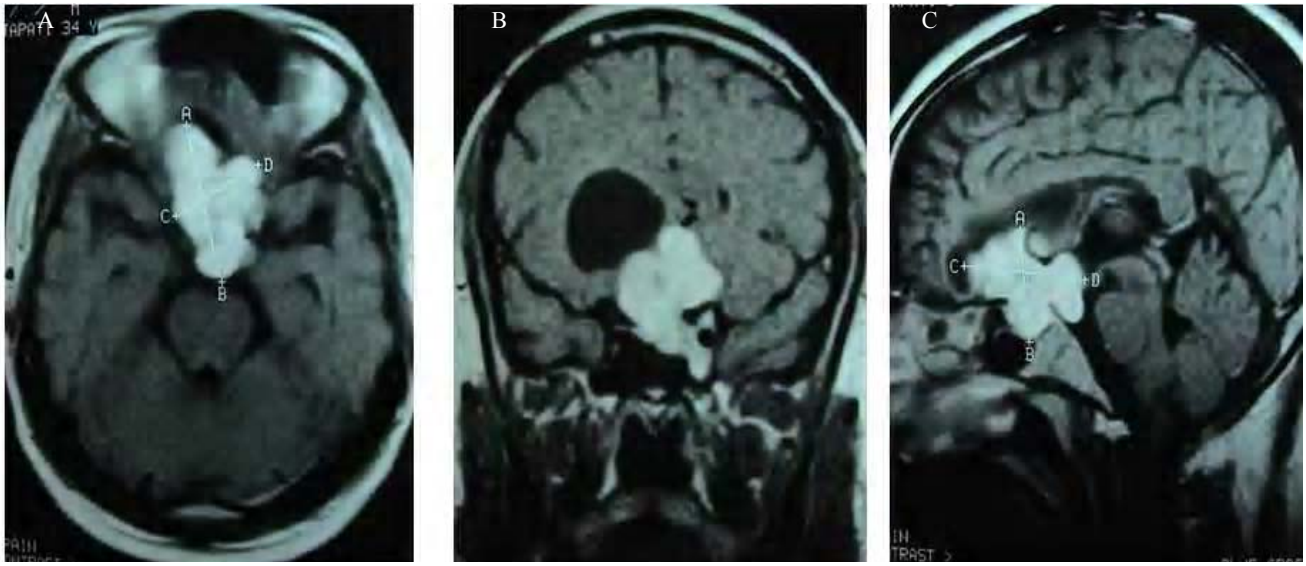

Figure 1. Magnetic Resonance Imaging (MRI) of the patient T1 contrast enhanced, A: Axial, B: Coronal and C: Sagittal sections showing sellar lesion with suprasellar extension

practice in the developing countries like ours. On the other hand, in advanced and developed countries, it's a routine procedure in the histopathological evaluation of any neoplastic lesion. As for example, if a brain tumor is resected and sent for histological evaluation, immunostaining is performed for various markers (protein component of tumor) along with other histological procedures like HE stain. Different tumor markers and diagnostic markers, according to the lesion, are then investigated. On that basis, a confirmed diagnosis is made and expected prognosis and clinical outcome is predicted depending upon which proper treatment is planned. It should be noted that despite the same histological diagnosis and grade, the outcome and prognosis significantly differs depending on several prognostic tumor markers. For example, Ki-67 (MIB I) is a marker protein, which reflects the cellular proliferation of any neoplastic lesion in the body.[1-3] Higher the Ki-67 expression higher is the cellular proliferation and thus poorer is the prognosis. The common tumor markers investigated in the histological evaluation of brain tumors are Ki-67, vemintin, S-100, glial fibrillary acidic protein (GFAP), Epidermal growth factor receptor (EGFR) etc.

Though brain is a comparatively small and narrow space in human body, varieties of neoplastic and non neoplastic lesions can develop in this region due to existence of varieties of histological structures. Tumors of sellar and parasellar region are sometimes difficult to differentiate from each other.[4-7] They often have similar clinical and radiological findings and sometimes they even resemble each other histologically. In such situation additional investigational tools become essential and helpful. Here we present a case of huge tumor of sellar region with suprasellar extension, provisionally diagnosed as a non functioning pituitary adenoma but was doubtful by its histological report. Immunohistochemistry was performed to make a final diagnosis.

Case Report

This 34-year-old male patient, referred from an eye hospital, presented with the gradual loss of vision on the left eye for more than a year with recent onset of progressive vision loss on the right side as well.0He also had features of raised ICP. On examination, he was fully conscious and had no focal neurological deficits. He had bitemporal hemianopia which was worse on the left side. Bilateral papilloedema was present. MRI showed huge heterogeneously enhanced intrasellar mass with a cystic component extending to supra and parasellar region compressing the 3rd ventricle **(Figure 1)**. On the basis of his clinical history and MRI findings the provisional diagnosis of non functioning pituitary macro-adenoma was made with a differential diagnosis of craniopharyngioma. Pituitary hormone status was within normal limit.

Surgery was performed. Almost complete excision of the

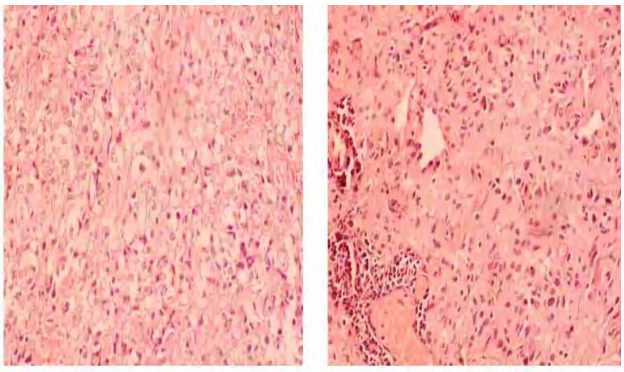

Figure 2. Histological slides of the case, HE stain, done after the excision of tumor

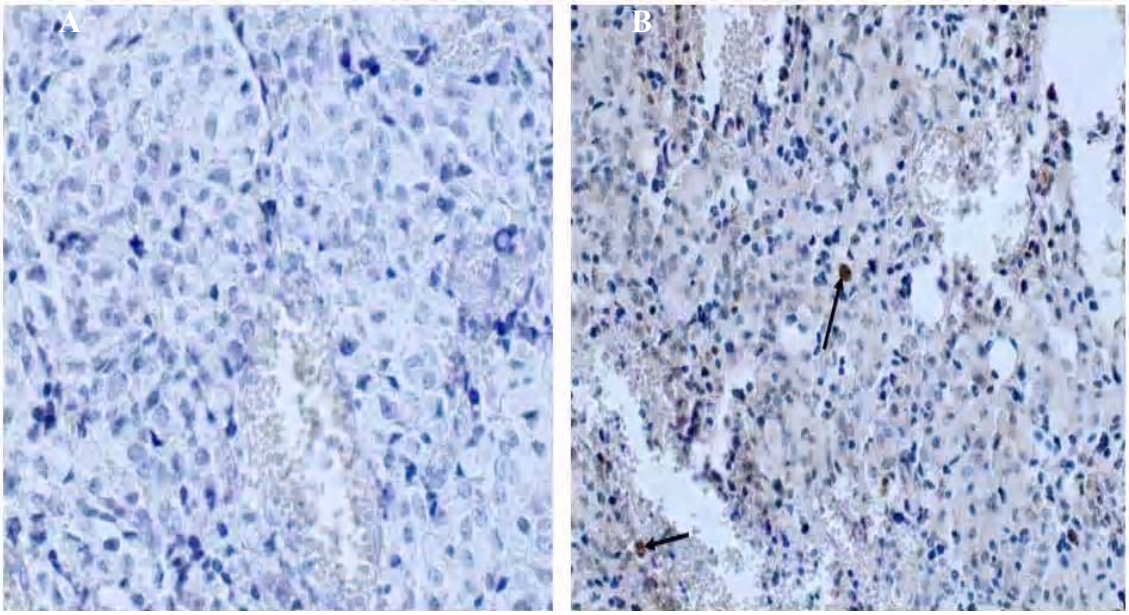

Figure 3.Immunohistochemical slides of the case, A: GFAP slide showing no positive cell under X 200 magnification, B: MIB-I slide showing very few positive cells (arrows), stained brown, under X 200 magnification

tumor was done with transcranial right frontal base approach. Tumor was soft in consistency and pale yellow in color.

The histological report of the tumor suggested that though the tumor looked like pituitary adenoma, some features of astrocytic or gliotic component suggesting astrocytoma or oligodendroglioma were also present **(Figure 2).** So the confirmed diagnosis couldn't be made. Thus immunostaining was planned and the tissue sample was sent to department of neurosurgery, Hiroshima University Hospital, where author did immunostaining of the formalin fixed paraffin embedded tissue section for GFAP, a protein originating from and a marker of glial, astrocytic, tissue [8], and Ki-67, a proliferation marker.[1,2]

Immunohistochemistry

The indirect method of immunostaining, LSAB (Labeled Strept Avidin Biotin method), was employed for antibody incubation using the histofine SAB kit produced by Nichirei Co. Tokyo, Japan [9]. Slides of 4 micrometer tissue sections were prepared and were deparaffinized with xylene, and antigen retrieval was done by the HIER (Heat Induced Epitope Retrieval) method using citrate buffer solution, pH 6.0. Endogenous peroxidase blocking was done by dipping the slides into a solution made by mixing 10ml of 30% H2O2 and 90 ml of methanol 99% for 30 min. After each step, the slides were rinsed and washed with PBS (phosphate buffer saline solution, pH 7.5) 3 times for 5 min each. The antibody used for GFAP was Clone 6F2, monoclonal mouse anti-human GFAP, DAKO, Denmark and was used at the dilution of 1:100. Similarly the primary antibody for Ki-67 was monoclonal mouse antibody, MIB-1, IMMUNOTECH, MARSEILLE, France and was used at the dilution of 1:50. Primary antibody incubation was performed overnight at 4° C, followed by secondary antibody incubation for 30 minutes. Secondary antibody was biotinylated secondary antibody, the SAB kit, derived from rabbit for both GFAP and Ki-67, produced by NICHIREI CO. TOKYO, JAPAN. Slides were treated with Mayer hematoxyline as a counterstain, for better cytoplasmic visualization, and then mounted with cover slips for storage purposes. The slides were evaluated for GFAP and Ki-67 (MIB-I) expression by the author and one of the co-authors.

Immunostaining Results

The slide picture of immunostaining is as shown in the **Figure 3.** The GFAP expression was negative suggesting that the tumor was not of glial (astrocytic) origin. Similarly, the MIB-I immunostaining showed very few positive cells, the labeling index being <1%. On the basis of these immunostaining features, possibility of astrocytic tumor was excluded.

Discussion

As has been already mentioned, immunostaining is a very essential clinical and research tool in the field of medical science. Many researches on tumor pathology are based on different marker proteins detected by immunostaining.

Diagnosis of pituitary adenoma can be made in various ways. Radiological information helps to a great extent in making provisional diagnosis. Histological diagnosis is the most precise as in case of other pathologies. However, sometimes histological picture alone can't be conclusive and thus some other information becomes essential to make a confirmed diagnosis. Immunohistochemistry is the one, which is very helpful in such situation by identifying the

lesion specific markers. In fact, histopathological evaluation of any lesion is incomplete without evaluation of such markers with the help of immunostaining.

Different markers can be searched for in case of pituitary adenoma. If the adenoma is the functional one, marker of each hormone, which the adenoma is producing, can be detected. For example, if the adenoma is growth hormone (GH) producing tumor, then we can detect the cells producing GH in the tumor using antibody against GH. Similarly other hormonal markers can also be detected by immunostaining. In this particular case, since pituitary hormones were normal and there were no other signs and symptoms suggesting hormonal hyperactivity, provisional diagnosis of non-functioning pituitary adenoma was made. The main difficulty here was to distinguish the tumor from astrocytoma. Therefore, we tried to detect GFAP, which is a marker protein of any glial tumor. GFAP was found to be negative suggesting the tumor didn't not have any glial component and thus was not of glial origin. We also tried to evaluate its progressiveness by evaluating MIB-I. A low expression of Ki-67 (MIB-I) also correlated with pituitary adenoma though it is possible in low grade astrocytoma as well.[10,11] Since the tumor was non-functioning, other hormonal markers were not evaluated by immunostaining. On the above basis, a final diagnosis of pituitary adenoma was made by excluding the possibility of astrocytic tumor. Intra-operative finding was another point in favor of pituitary adenoma.

However, there was a limitation in this case. We could have done immunostaining even for other hormonal markers to detect if at all there was any hormonal activity. Absence of all the hormonal markers would suggest that the tumor is non-functioning. Similarly, detection of hormonal marker by immunohistochemistry in a functioning pituitary tumor is more confirmatory for diagnosis.

References

1. Suzuki M, Minematsu T, Oyama K et al: Expression of proliferation markers in human pituitary incidentalomas. **Endocr Pathol 17:**263-75, 2006
2. Giannini C, Scheithauer BW, Burger PC et al: Cellular proliferation in pilocytic and diffuse astrocytomas. **J Neuropathol Exp Neurol 58(1):**46-53, 1999
3. Ferreira JE, de Mello PA, de Magalhães AV et al: Non-functioning pituitary adenomas: clinical features and immunohistochemistry. **Arq Neuropsiquiatr 63(4):**1070-8, 2005
4. Moreno-Perez O, Peiró FM, López P et al: An isolated pituitary metastasis as presentation of a differentiated hepatocellular carcinoma mimicking a nonfunctioning macroadenoma. **J Endocrinol Invest 30(5):** 428-33, 2007
5. Rennert J, Doerfler A: Imaging of sellar and parasellar lesions. **Clin Neurol Neurosurg. 109(2):**111-24, 2007
6. Yilmazlar S, Bekar A, Taskapilioglu O et al: Isolated intrasellar tuberculoma mimicking pituitary adenoma. **J Clin Neurosci 14(5):** 477-81, 2007
7. Bisson E, Khoshyomn S, Braff S et al: Hypothalamic-opticochiasmatic gliomas mimicking craniopharyngiomas. **Pediatr Neurosurg. 39(3):**159-65, 2003
8. USBiological, Growth factors, cytokines, GFAP, http://www.usbio.net/Product.aspx?ProdSku=G2032-28B. [Accessed on 22, September, 2007]
9. Shrestha P, Saito T, Hama S et al: Geminin: a good prognostic factor in high-grade astrocytic brain tumors. **Cancer109(5):** 949-56, 20070
10. Mahta A, Haghpanah V, Lashkari A et al: Non-functioning pituitary adenoma: immunohistochemical analysis of 85 cases. **Folia Neuropathol 45(2):** 72-7, 2007
11. Losa M, Franzin A, Mangili F et al: Proliferation index of nonfunctioning pituitary adenomas: correlations with clinical characteristics and long-term follow-up results. **Neurosurgery 47(6):**1313-8, 2000